REMARKS UNDER 37 CFR § 1.116

Formal Matters

Claims 30, 31 and 33 are now pending in this application, following the present amendments.

Claims 30, 31 and 33 have been amended to more particularly point out and distinctly claim the invention. The amendments to the claims were made solely in the interest of expediting prosecution, and are not to be construed as an acquiescence to any objection or rejection of any claim.

Please replace claims 30, 31 and 33 with the clean version provided above.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

Applicants respectfully request reconsideration of the application in view of the remarks made herein.

Information Disclosure Statement

At the Examiner's request, Applicants have resubmitted the Information Disclosure Statement and copies of references filed on October 2, 2000.

Applicants respectfully request that the Examiner initial and return the PTO/SB0/8A form submitted with the Information Disclosure Statement filed on October 2, 2000 in this application, thereby indicating that the references cited therein have been reviewed and made of record.

Supplemental Information Disclosure Statement

A Supplemental Information Disclosure Statement, including a Form PTO/SB0/8A and copies of a patent are submitted in this application herewith. Consideration of the references cited therein is respectfully requested.

Applicants respectfully request that the Examiner initial and return the PTO/SB0/8A form submitted with this Supplemental Information Disclosure Statement, thereby indicating that the references cited therein have been reviewed and made of record.

Telephone interview

Applicants wish to extend their gratitude to Examiner Ly for a telephone interview with Applicants' representitive James S. Keddie on November 8, 2002.

The enablement rejection was clarified by Examiner Ly.

The Response in General

Claims have been rejected for lack of enablement.

Applicants have provided an enabling disclosure for what they are claiming. Specifically, applicants have provided methods for crystallizing a total of 4 NS5B HCV polymerase polypeptides, and have provided high resolution atomic coordinates for all four crystals. All one of skill in the art would have to do to make further HCV polymerase crystals is merely perform the described methods. Also, since the provided atomic coordinates show that the structures of the HCV polymerases are similar, one of skill in the art would be able to reliably identify an NS5B HCV polymerase inhibitor without any undue experimentation. Furthermore, crystal structures for the same molecules have subsequently been published in several journals, demonstrating that one of skill in the art could make further NS5B HCV polymerase crystals and resolve their atomic coordinates. Based on these observations alone, the first rejection based on lack of enablement should be withdrawn.

To the extent a further discussion is believed necessary, the Examiner is respectfully referred to the following.

Rejections under 35 U.S.C. §112, first paragraph (enablement)

Claims 30, 31 and 33 were rejected under 35 U.S.C. § 112, first paragraph, for containing subject matter which was not described in such a way as to enable one of skill in the art to make and use the invention commensurate in scope with these claims. Specifically, the Office Action asserts that while the specification is enabling for an HCV polymerase which have the disclosed coordinates, the specification does not reasonable provide enablement for an HCV polymerase inhibitor.

The Office Action acknowledges that the applicant has disclosed information to enable one skilled in the art to make a crystal of the HCV polymerase using SEQ ID NO:2 of $NS5B_{570}$, and asserts that one skilled in the art would a) not be able to use the disclosed information to make other NS5B crystals of predictable quality, and b) not be able to use the disclosed information regarding $NS5B_{570}$ structure to reliably predict the three-dimensional structure of a test compound without actually generating a crystal

structure of the test compound.

The law is clear that "[t]he test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation."[1] The Office is reminded that extensive experimentation may be performed, so long as the experimentation is routine, and that every species within a genus does not have to be operative for a claim to be fully enabled.

The Applicants respectfully submit that the specification and the amended claims, coupled with the information known in the art, would enable one of skill in the art to use the invention without undue experimentation.

As the Office Action pointed out, a major factor in determining whether a claim is enabled is the presence of absence of working examples.

In their working examples applicants have provided methods for crystallizing 4 different NS5B derivatives, including $NS5B_{570}$ (see Example 2, pages 22, line 21 to page 23, line 6), $NS5B_{544}$, $NS5B_{536}$, and $NS5B_{531}$ (see Example 2, page 23, lines 8 to 16). $NS5B_{544}$, $NS5B_{536}$, and $NS5B_{531}$ were crystallized using an identical method.

Furthermore, Applicants have provided high resolution atomic coordinates for all four derivatives. The crystal structure of $NS5B_{570}$ is described in Example 3, on page 23, line 24 to page 25, line 12, and the atomic coordinates are provided in Table 2. The crystal structures of $NS5B_{544}$, $NS5B_{536}$, and $NS5B_{531}$ are described in Example 3, on page 25 lines 13-16, and the atomic coordinates for $NS5B_{544}$, to which the atomic coordinates of $NS5B_{536}$, and $NS5B_{531}$ are similar, are provided in Table 3.

Applicants assert that these examples enable the full scope of the claims.

As the Applicants understand the rejection, the Office has implied that one of skill in the art would not be able to use the atomic coordinates provided in the instant specification to identify an inhibitor.

Applicants assert that this is not so. Each protein molecule has only one crystal structure, and one associated set of atomic coordinates. Once a set of atomic coordinates for a single crystal is provided, one of skill in the art can reliably use the coordinates as an accurate description of the structure of the crystallized molecule. If this were not the case, what would be the point of publishing a crystal structure?

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[1] *United States v. Telectronics, Inc.*, 8 USPQ 2d 1217, 1233 (Fed. Cir. 1988), *cert. denied*, 490 U.S. 1046 (1989). *See also, Genentech, Inc. v. Novo Nordisk*, 42 USPQ 2d 1001 (Fed. Cir. 1997), *cert. denied*, 522 U.S. 963 (1997); *Scripps Clinic and Research Foundation v. Genentech, Inc.*, 18 USPQ 2d 1001 (Fed. Cir. 1991).

Furthermore, the atomic coordinates of $NS5B_{544}$, $NS5B_{536}$, and $NS5B_{531}$, as stated above, are all similar, giving one of skill in the art even greater confidence that he could use the atomic coordinates provided in the specification in the claimed method. As such, one of skill in the art would certainly use the provided atomic coordinates, which are likely to be like atomic coordinates of other NS5B derivatives, in performing the claimed methods without any undue experimentation.

The Office has cited "J. Drenth" to show that crystallization is a trial-and-error method and the results are usually unpredictable. Drenth is referring to an idea that one of skill in the art, given a purified polypeptide, would not be able to predict which crystallization conditions to use. However, once a suitable crystallization method is worked out for a polypeptide, one of skill in the art would be able to use the worked out method to crystallize the polypeptide or similar polypeptides with no undue experimentation.

Applicants assert that one of skill in the art, if they wanted to do so, would be able to able to crystallize a NS5B polymerase recited in the claims using the provided methods without any undue experimentation. Since the inventors crystallized several different NS5B derivatives independently under the same conditions, and these conditions are provided in exceptional detail in example 2 of the specification, one of skill in the art would merely have to repeat the methods to provide suitable crystals.

Publications subsequent to the instant priority date further demonstrate that one of skill in the art could routinely crystallize and resolve the subject polymerase. These publications, abstracts of which are enclosed herewith, include:

Ago et al., *Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus.* Structure Fold Des. (1999) 7:1417-26;

Bressanelli et al., *Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus.* Proc Natl Acad Sci U S A. (1999) 96:13034-9; and

Lesburg et al., *Crystal structure of the RNA-dependent RNA polymerase from hepatitis C virus reveals a fully encircled active site.* Nat Struct Biol. (1999) 6:937-43.

All studies, including those of the instant application, show very similar structures for the subject polymerase.

As such, one of skill in the art would be able to practice the claimed methods to the full scope of the claims without undue experimentation. Accordingly the rejection of these claims under 35 U.S.C. §112 paragraph 1 (enablement) should be withdrawn.

The Office has further rejected claim 33 under 35 U.S.C. §112 for the asserted reason that the

specification is not enabling for determining HCV polymerase activity via reacting a template RNA and substrates in the absence of a test compound and comparing the HCV polymerase activity. The Office acknowledges that the applicant has disclosed information to enable one skilled in the art to determine activity of HCV polymerase in the presence of a test compound.

Applicants assert that comparing enzyme activity in the presence of an agent to enzyme activity in the absence of the agent is a very well known method in drug discovery, and, as such, one of skill in the art would be able to perform such methods without undue experimentation. The method is very straightforward: assay a protein's activity in the presence and in the absence of an agent and determine whether the agent causes a relative reduction in the activity of the protein. The activity is usually expressed as a percentage or fraction of the activity in the absence of the agent. These methods are standard for drug screening.

Furthermore, applicants have used this methods in their analysis of HCV polymerase activity. In Example 7 of the instant application, on page 281, line 34 to page 282, line 6 of the specification, a description of an experiment where polymerase activities are measured with and without a synthetic peptide and the results compared. The Example states, on page 282 lines 5-6 that "The synthetic peptide inhibited the polymerase activity 40-50% at a final concentration of 30 μm", and as such, a comparison between the two polymerase activities has been made.

As such, the specification provides an enabling disclosure for a claim that recites determining HCV polymerase activity via reacting a template RNA and substrates in the absence of a test compound and comparing the HCV polymerase activity. Accordingly the rejection of claim 33 under 35 U.S.C. §112 (enablement) should be withdrawn.

Rejections under 35 U.S.C. §112, second paragraph (indefiniteness)

Claims 30, 31 and 33 are rejected under 35 U.S.C. §112 as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicants have addressed these rejections by amending the claims. The preambles of claims 30 and 31 are amended to "A method for identifying a HCV polymerase inhibitor", rather than a "A method for designing or identifying HCV polymerase inhibitors. Furthermore, the applicants have added a "wherein" clause to each of the claims, which allows the claims accomplish their intended methods.

As such, applicants assert that this rejection has been addressed and the rejection of claims 30, 31 and 33 under 35 U.S.C. §112, second paragraph (indefiniteness) should be withdrawn.

Conclusion

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number SHIM006.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 12-06-2002

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS

Claims 30, 31 and 33 are amended, as shown below.

30. (Amended) A method for ~~designing or~~ identifying a HCV polymerase inhibitors, ~~which~~ said method comprising: ~~comprises~~

determining the complementarity of a test compound with an active site and/or RNA binding cleft of a polypeptide using ~~the~~ a three-dimensional structural coordinate of said polypeptide or its part and ~~the~~ a three-dimensional structural coordinate of ~~the~~ said test compound,

wherein said polypeptide is derived from ~~the~~ an NS5B HCV polymerase, ~~NS5B having~~ has an NS5B HCV polymerase activity, and ~~consisting~~ consists of an amino acid sequence X-Y, wherein X is a consecutive amino acid sequence which is a portion of ~~the~~ NS5B, ~~an~~ the N-terminal amino acid of X is a serine residue corresponding to ~~the~~ amino acid residue 1 ~~(Ser)~~ of ~~the~~ NS5B, and the ~~a~~ C-terminal amino acid residue of X is any one of amino acid residues 531(Lys) to 570 (Arg) of ~~the~~ NS5B; and wherein Y is a carboxyl group or ~~another~~ an amino acid sequence which is not derived from NS5B; and wherein one or more amino acids in X may be modified, and wherein methionine residues in the amino acid sequence of X may be replaced by selenomethionine residues,

wherein a test compound that is complementary to said active site and/or RNA binding cleft of said polypeptide is a HCV polymerase inhibitor.

31. (Amended) A method for ~~designing or~~ identifying a HCV polymerase inhibitors, which method comprises the steps of:

~~(a) determining the complementarity of a test compound with an active site and/or RNA binding cleft of a polypeptide using a three-dimensional structural coordinate of said polypeptide or its part and a three-dimensional structural coordinate of said test compound, wherein said polypeptide is derived from the HCV polymerase NS5B having an HCV polymerase activity and consisting of an amino acid sequence X-Y, wherein X is a consecutive amino acid sequence which is a portion of the NS5B, an N-terminal amino acid of X is the amino acid residue 1 (Ser) of the NS5B, and a C-terminal amino acid~~

~~residue of X is any one of amino acid residues 531 (Lys) to 570 (Arg) of the NS5B; and~~

~~wherein Y is a carboxyl group of another~~ an ~~amino acid sequence which is not derived from NS5B;~~ and

~~wherein one or more amino acids in X may be modified, and methionine residues in the amino acid sequence of X may be replaced by selenomethionine residues;~~

(a) performing the method of claim 30; and

(b) determining a HCV polymerase-inhibitory activity of said HCV polymerase inhibitor. ~~test compound; and~~

~~(c) designing or determining HCV polymerase inhibitors using the complementarity data of said test compound determined in the above (a), and the inhibitory activity data obtained in the above (b).~~

33. (Amended) A method for identifying a HCV polymerase inhibitors, which method comprises the steps of:

(a) obtaining a polypeptide, which is derived from ~~the~~ an NS5B HCV polymerase, ~~NS5B~~ has an NS5B HCV polymerase activity, and ~~consisting~~ consists of the amino acid sequence X'-Y, wherein X' is a consecutive amino acid sequence which is a portion of the NS5B, ~~an~~ the N-terminal amino acid of X' is a serine residue corresponding to ~~the~~ amino acid residue 1 ~~(Ser)~~ of ~~the~~ NS5B, ~~a~~ and the C-terminal amino acid residue of X' is any one of amino acid residues 531 (Lys) to 544 (Gln) of ~~the~~ NS5B; and wherein Y is a carboxyl group or another amino acid sequence which is not derived from NS5B; and wherein one or more amino acids in X' may be modified, and methionine residues in the amino acid sequence of X' may be replaced by selenomethionine residues;

(b) determining the HCV polymerase activity of said polypeptide by reacting said polypeptide obtained in step ~~the above~~ (a) with a template RNA and substrates in the presence of a test compound;

(c) determining the HCV polymerase activity of said polypeptide by reacting polypeptide obtained in step ~~the above~~ (a) with a template RNA and substrates in the absence of said test compound; and,

(d) comparing the HCV polymerase activity determined in step ~~the above~~ (b) with the HCV polymerase activity determined in step ~~of the above~~ (c),

wherein an activity determined in step (b) that is lower than the HCV polymerase activity determined in step (c) indicates that the test agent is an HCV polymerase inhibitor.

1: Proc Natl Acad Sci U S A 1999 Nov 9;96(23):13034-9 Related Articles, Links

Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus.

Bressanelli S, Tomei L, Roussel A, Incitti I, Vitale RL, Mathieu M, De Francesco R, Rey FA.

Virologie Moleculaire Structurale, Laboratoire de Genetique des Virus, Centre National de la Recherche Scientifique/Unite Propre de Recherche 9053 1, Avenue de la Terrasse, F-91198 Gif-sur-Yvette Cedex, France.

We report the crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus, a major human pathogen, to 2.8-A resolution. This enzyme is a key target for developing specific antiviral therapy. The structure of the catalytic domain contains 531 residues folded in the characteristic fingers, palm, and thumb subdomains. The fingers subdomain contains a region, the "fingertips," that shares the same fold with reverse transcriptases. Superposition to the available structures of the latter shows that residues from the palm and fingertips are structurally equivalent. In addition, it shows that the hepatitis C virus polymerase was crystallized in a closed fingers conformation, similar to HIV-1 reverse transcriptase in ternary complex with DNA and dTTP [Huang H., Chopra, R., Verdine, G. L. & Harrison, S. C. (1998) Science 282, 1669-1675]. This superposition reveals the majority of the amino acid residues of the hepatitis C virus enzyme that are likely to be implicated in binding to the replicating RNA molecule and to the incoming NTP. It also suggests a rearrangement of the thumb domain as well as a possible concerted movement of thumb and fingertips during translocation of the RNA template-primer in successive polymerization rounds.

PMID: 10557268 [PubMed - indexed for MEDLINE]

1: Nat Struct Biol 1999 Oct;6(10):937-43

Nature Structural Biology

Crystal structure of the RNA-dependent RNA polymerase from hepatitis C virus reveals a fully encircled active site.

Lesburg CA, Cable MB, Ferrari E, Hong Z, Mannarino AF, Weber PC.

Department of Structural Chemistry, Schering-Plough Research Institute, Kenilworth, New Jersey 07033, USA.

Various classes of nucleotidyl polymerases with different transcriptional roles contain a conserved core structure. Less is known, however, about the distinguishing features of these enzymes, particularly those of the RNA-dependent RNA polymerase class. The 1. 9 A resolution crystal structure of hepatitis C virus (HCV) nonstructural protein 5B (NS5B) presented here provides the first complete and detailed view of an RNA-dependent RNA polymerase. While canonical polymerase features exist in the structure, NS5B adopts a unique shape due to extensive interactions between the fingers and thumb polymerase subdomains that serve to encircle the enzyme active site. Several insertions in the fingers subdomain account for intersubdomain linkages that include two extended loops and a pair of antiparallel alpha-helices. The HCV NS5B apoenzyme structure reported here can accommodate a template:primer duplex without global conformational changes, supporting the hypothesis that this structure is essentially preserved during the reaction pathway. This NS5B template:primer model also allows identification of a new structural motif involved in stabilizing the nascent base pair.

PMID: 10504728 [PubMed - indexed for MEDLINE]

1: Structure Fold Des 1999 Nov 15;7(11):1417-26

Related Articles, Links

Full Text on BioMedNet GO

Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus.

Ago H, Adachi T, Yoshida A, Yamamoto M, Habuka N, Yatsunami K, Miyano M.

Central Pharmaceutical Research Institute, Japan Tobacco Inc., Takatsuki, 569-1125, Japan.

BACKGROUND: Hepatitis C virus (HCV) is the major etiological agent of hepatocellular carcinoma, and HCV RNA-dependent RNA polymerase (RdRp) is one of the main potential targets for anti-HCV agents. HCV RdRp performs run-off copying replication in an RNA-selective manner for the template-primer duplex and the substrate, but the structural basis of this reaction mechanism has still to be elucidated. RESULTS: The three-dimensional structure of HCV RdRp was determined by X-ray crystallography at 2.5 A resolution. The compact HCV RdRp structure resembles a right hand, but has more complicated fingers and thumb domains than those of the other known polymerases, with a novel alpha-helix-rich subdomain (alpha fingers) as an addition to the fingers domain. The other fingers subdomain (beta fingers) is folded in the same manner as the fingers domain of human immunodeficiency virus (HIV) reverse transcriptase (RT), another RNA-dependent polymerase. The ribose-recognition site of HCV RdRp is constructed of hydrophilic residues, unlike those of DNA polymerases. The C-terminal region of HCV RdRp occupies the putative RNA-duplex-binding cleft. CONCLUSIONS: The structural basis of the RNA selectivity of HCV RdRp was elucidated from its crystal structure. The putative substrate-binding site with a shallow hydrophilic cavity should have ribonucleoside triphosphate (rNTP) as the preferred substrate. We propose that the unique alpha fingers might represent a common structural discriminator of the template-primer duplex that distinguishes between RNA and DNA during the replication of positive single-stranded RNA by viral RdRps. The C-terminal region might exert a regulatory function on the initiation and activity of HCV RdRp.

PMID: 10574802 [PubMed - indexed for MEDLINE]